INHIBITION OF BACTERIA BY 5-FLUORONICOTINIC ACID AND OTHER ANALOGUES OF NICOTINIC ACID

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ABSTRACT

STREIGHTOFF, FRANK (The Lilly Research Laboratories, Indianapolis, Ind.). Inhibition of bacteria by 5-fluoronicotinic acid and other analogues of nicotinic acid. J. Bacteriol. **85**:42–48. 1963.—Several compounds related to 5-fluoronicotinic acid (5-FNA) have been demonstrated to inhibit *Streptococcus* sp. (Viridans group), *Staphylococcus aureus*, *Escherichia coli*, and *Lactobacillus plantarum* in vitro. The most active compounds were 5-FNA and 5-fluoronicotinamide (5-FNAM). The growth of *Streptococcus* sp. was inhibited more than 50% by 0.05 μg/ml of 5-FNA or 0.5 μg/ml of 5-FNAM. The inhibition of *Streptococcus* sp. from 1 part of 5-FNA or 5-FNAM was reversed by 4 and 2 parts of nicotinic acid, respectively. The inhibition of *E. coli* from 100 parts of 5-FNA or 5-FNAM was reversed by 1 part of nicotinic acid. Inhibitions by most other active compounds could be reversed by nicotinic acid. In experiments with mice, eight compounds related to 5-FNA had activity against *Streptococcus pyogenes*; 5-FNA, 5-FNAM, and 5-fluoro-*N*-dimethylaminomethylnicotinamide protected all mice at 83 mg/kg × two treatments subcutaneously. The action of 200 mg/kg × two treatments of 5-FNA was reversed by 20 mg/kg × two treatments of nicotinic acid. The activity of 5-FNA was not increased by modifications at the number 3 or 5 positions on the pyridine ring or by any other structural changes.

Fildes (1940) postulated the competitive nature of the relationship between the metabolite *p*-aminobenzoic acid and sulfonamides in certain bacteria. The clinical success of the sulfa drugs led to the hope that additional chemotherapeutic agents acting competitively against other bacterial metabolites could be found. In pursuit of this objective, many analogues of bacterial growth factors have been tested. In 1948, 5-fluoronicotinic acid (5-FNA) and 5-fluoronicotinamide (5-FNAM) were tested in these laboratories for activity (Hawkins and Roe, 1949). These compounds were found to be highly competitive for nicotinic acid. At that time, a number of additional analogues were examined. More recently, a new series of analogues has been studied. This paper describes the in vitro and in vivo experiments with these compounds.

Nicotinic acid or nicotinamide is essential for the growth or stimulates the growth of a large number of microorganisms (Porter, 1946; Peterson and Peterson, 1945). Some closely related compounds also have nicotinic acidlike activity (Porter, 1946). McIlwain (1940) reported that 3-pyridine sulfonamide inhibited the growth of *Staphylococcus aureus*, and that this inhibition could be reversed by nicotinamide. Schmidt-Thomé (1948) found that the inhibition of *Staphylococcus* by 6-aminonicotinic acid could be reversed by nicotinic acid. Hughes (1954) investigated the antinicotinic acid activity of five halonicotinic acids. He found that these acids inhibited the growth of *Lactobacillus arabinosus*, *S. aureus*, *Proteus vulgaris*, and *Escherichia coli* in the following order of effectiveness: 5-FNA, 5-chloronicotinic acid, 5-bromonicotinic acid, 2-fluoronicotinic acid, and 6-fluoronicotinic acid. He demonstrated that the inhibition was reversed competitively by nicotinic acid, nicotinamide, and diphosphopyridine nucleotide (DPN). DPN synthesis by *L. arabinosus* was inhibited by 5-fluoronicotinic acid. The acceleration of glycolysis in *L. arabinosus* and *S. aureus* by nicotinic acid was also inhibited by 5-fluoronicotinic acid.

P. J. Simpson (*personal communication*) examined 5-fluoronicotinic acid and several related compounds for inhibition of the growth of *Bacillus subtilis* and *Euglena*, using agar diffusion procedures. In order of their activity, the first five compounds were: 5-FNA, 5-FNAM, 5-fluoro-*N'*-dimethylaminomethylnicotinamide, 5-fluoronicotinhydroxamic acid, and 5-methyl-

N'-dimethylaminomethylnicotinamide. Agents which reversed the inhibition of both *B. subtilis* and *Euglena* by 5-FNA were nicotinamide, nicotinic acid, DPN, and triphosphopyridine nucleotide.

Burchenal et al. (1959) found that 200 to 250 mg/kg of 5-FNAM administered intraperitoneally daily for 10 to 14 days brought about 83 to 91% and 52% inhibition, respectively, of leukemias B82T and P815 in mice. This inhibition could be prevented by the concurrent administration of nicotinamide. I. S. Johnson (*personal communication*) found a similar response with leukemia strain B82 in mice. Surprisingly, 5-FNAM had an activity equivalent to nicotinamide in reversing the inhibition of leukemia B82 by 2-amino-1,3,4-thiadiazole (Oettgen et al., 1960).

Materials and Methods

Organisms. Earlier experiments were conducted with *Streptococcus* sp. (Viridans group) strain 1820, *Staphylococcus aureus* strain 1041, and *E. coli* strain 105. The present study was conducted with *Streptococcus* sp. strain 1820, *L. plantarum* strain ATCC 8014 (used for nicotinic acid assays), and *E. coli* strain ATCC 8723b (mutant requiring niacin). The in vivo experiments were performed in Webster Swiss strain of white mice infected with either *E. coli* strain 0127, *Proteus vulgaris* strain ATCC 9484, *Pseudomonas aeruginosa* strain X-239, or *Streptococcus pyogenes* strain C-203. *S. pyogenes* strain C-203 was obtained from L. H. Schmidt of the Medical School, University of Cincinnati; ATCC strains of organisms were obtained from the American Type Culture Collection; all other strains were obtained from the Lilly culture collection.

In vitro methods. The effect of analogues of nicotinic acid was studied in a chemically defined medium containing minimal amounts of nicotinic acid for the various test organisms. In the earlier study, *Streptococcus* sp. strain 1820 was repeatedly transferred until it grew luxuriantly in a medium of amino acids, purines, vitamins, minerals, glucose, and sodium acetate (Greenhut, Schweigert, and Elvehjem, 1946). *S. aureus* strain 1041 was adapted to growth in this same medium. *E. coli* strain 105 grew with no difficulty in the simple medium which contained only NaCl, $(NH_4)_2SO_4$, K_2HPO_4, $MgSO_4$, glucose, $FeSO_4 \cdot 6H_2O$, and L-asparagine (MacLeod, 1940).

In the present study, the stock culture of *Streptococcus* sp. strain 1820 could not be readapted to grow in Greenhut's medium. A modified medium in which the amino acids were replaced by Casamino Acids (Difco), tryptophan, and L-cystine was used. The previously used strains of *S. aureus* and *E. coli* were no longer available. Instead, *L. plantarum* strain ATCC 8014 was grown in Niacin Assay Medium, and *E. coli* strain ATCC 8723b was grown in MacLeod's medium.

The effect of 5-FNA and related compounds on the growth of these test organisms was studied. During the first series, turbidity readings were made with a Klett-Summerson colorimeter. During the second series, turbidity, owing to growth of the organism in its medium with a minimal amount of added nicotinic acid, was determined at 550 mμ on a Coleman Junior spectrophotometer.

If the addition of a compound to the medium resulted in a lower turbidity than the control, the reduction was then calculated in terms of percentage inhibition. When a compound was found to have inhibitory action, smaller quantities were added in an attempt to determine the magnitude of the activity. If compounds were found to inhibit growth of one or more organisms, increased amounts of nicotinic acid were added in an effort to reverse the inhibition. Compounds which did not inhibit growth were checked for nicotinic acid activity.

In vivo methods. When an adequate amount of compound was available, in vivo tests were carried out against mice, as previously described (Wick, Streightoff, and Holmes, 1961). Mice were infected intraperitoneally with dilutions of 18-hr cultures. Compounds were administered by the subcutaneous route 1 and 5 hr after infection. The days of survival of the mice were recorded through the 7th day after infection. Antibacterial activity of each compound was judged on the basis of prolongation of life and the number of survivors. In the case of 5-FNAM, reversal of the protection afforded by this compound against *S. pyogenes* was attempted by the administration of nicotinamide at a different site.

Results

In vitro tests with Streptococcus sp. (Viridans group) strain 1820. Of a total of 43 compounds

TABLE 1. *Inhibition of bacterial growth by 5-fluoronicotinic acid and related compounds and its reversal by nicotinic acid*

Compound	First series								
	Streptococcus sp. 1820			*Staphylococcus aureus* 1040			*Escherichia coli* 105		
	Inhibition	μg/5 ml	Reversal	Inhibition	μg/5 ml	Reversal	Inhibition	μg/5 ml	Reversal
	%			%			%		
5-Bromonicotinamide	0	2,500		0	2,500		100	2,500	Yes
5-Bromonicotinic acid	0	2,500		0	2,500		30	2,500	
5-Chloronicotinic acid	0	2,500		0	2,500		35	2,500	
5,6-Dichloronicotinic acid	100	1,250	Yes	0	2,500		35	2,500	
5-Fluoronicotinamide (5-FNAM)	90	2.5	Yes	80	100	Yes	100	5	Yes
	100*	25		0*	5,000		0*	250	
5-Fluoronicotinic acid (5-FNA)	90	0.25	Yes	0	2,500		80	5	Yes
	100*	25					0*	250	
5-Fluoronicotinic acid, sodium salt							95	2.5	Yes
5-Iodonicotinamide	80	2,500		0	2,500		50	2,500	Yes
5-Iodonicotinic acid	0	2,500		0	2,500		40	2,500	
Methyl-5-bromonicotinate	100	156	Yes	100	156	Yes	100	2,500	
Methyl-5-fluoronicotinate	100	2.5	Yes	50	1,250	Yes	100	25	Yes

Compound	Second series								
	Streptococcus sp. 1820			*Lactobacillus plantarum* ATCC 8014			*E. coli* ATCC 8723b		
	%			%			%		
5-Aminonicotinamide·HCl				0	250				
4-Aminonicotinic acid	70	1,000		0	1,000		0	1,000	
5-Aminonicotinic acid	0	2,500							
5-Aminonicotinic acid·HCl	40	2,500		0	500		0	500	
	0	250							
6-Aminonicotinic acid	100	2,500	Yes	0	250	Yes	100	250	Yes
	40	250					70	25	
5,6-Diaminonicotinic acid				0	250				
5-Fluoro-3-acetylpyridine·HCl				0	250				
5-Fluoro-3-cyanopyridine	100	2,500	Yes	0	2,500		100	2,500	Yes
							50	250	
5-Fluoro-N^1-dimethylaminomethylnicotinamide	100	25	Yes	100	2,500		100	25	Yes
	50	2.5		50	250				
5-Fluoro-*N*,*N*-dimethylnicotinamide	50	2,500							
6-Fluoronicotinamide	0	2,500		0	500		0	500	
5-Fluoronicotinamide-*N*-oxide	100	2,500							
5-Fluoronicotinic acid (5-FNA)	100	1		70	1,000	Yes	100	1	Yes
				60	100		60	0.1	
6-Fluoronicotinic acid	0	2,500							
5-Fluoronicotinic acid hydrazide	100	50	Yes	0	500		100	500	Yes
	50	5					40	50	
5-Fluoronicotinic acid-*N*-oxide	90	2,500							
5-Fluoronicotinhydroxamic acid	90	250	Yes	60	1,000	Yes	100	250	Yes
							40	25	
5-Fluoronicotinuric acid	100	250		0	250		25	250	
	60	25							
5-Fluoroquinolinic acid, monosodium salt	30	2,500							
5-Fluorothionicotinamide	100	100	No	0	250		0	250	

TABLE 1. (*Cont.*)

Compound	Second series								
	Streptococcus sp. 1820			*Lactobacillus plantarum* ATCC 8014			*E. coli* ATCC 8723b		
	%			%			%		
5-Hydroxynicotinic acid	50	2,500							
5-Methylnicotinamide	0	2,500							
4-Methylnicotinic acid	80	2,500		0	250				
	0	100							
5-Methylnicotinic acid	0	2,500		0	2,500		50	2,500	
5-Methylthionicotinamide	100	100	No	0	2,500				
Nicotinhydroxamic acid	0	2,500		0	2,500		100	2,500	No
4-Nitronicotinic acid-*N*-oxide	100	2,500		0	2,500		100	2,500	
5-Phenylnicotinic acid	0	2,500		0	2,500		30	2,500	
5-Pyrimidine carboxylic acid	0	2,500		0	2,500		0	2,500	
5-Thiazole carboxamide	0	250		0	250		100	25	Yes
							70	2.5	
5-Thiazole carboxylic acid	100	250	No	0	2,500		30	2,500	
Thioisonicotinamide	100	100	No	0	2,500		0	2,500	
	75	25							
Thionicotinamide	100	100	No	0	2,500		0	2,500	

* Nutrient broth was used instead of the regular medium. It has nicotinic acid activity.

investigated by in vitro means, 39 were tested against *Streptococcus*. Of these compounds, 26 showed inhibitory action against growth, and 24 of these showed inhibition of 50% or more (Table 1). All but 7 of these compounds had a substitution in the 5 position of the pyridine ring; 16 of the compounds inhibiting 50% or more were checked for reversal by nicotinic acid, and the inhibition of 11 was reversed. All of the inhibiting compounds reversed, except 6-aminonicotinic acid, had a substitution in the 5 position. The inhibition of 5-fluorothionicotinamide and 5-methylthionicotinic acid was not reversed. Other compounds not reversed by nicotinic acid addition were 5-thiazole carboxylic acid, thionicotinic acid, and thioisonicotinamide.

The most active compound was 5-FNA; 0.05 μg/ml of this compound inhibited the growth of *Streptococcus* by 90%. It required 0.2 μg/ml of nicotinic acid to reverse the inhibitory action of 0.05 μg/ml of 5-FNA (Table 2). The next most active compounds were 5-FNAM and methyl-5-fluoronicotinate, with activity at 0.5 μg/ml. It required 1 μg/ml of nicotinic acid to reverse the inhibition of 0.5 μg/ml of 5-FNAM.

Tests with S. aureus strain 1041. Of ten analogues of nicotinic acid tested, only three showed 50% or more inhibition. These compounds all had substitutions in the 5 position. All were reversed by nicotinic acid. 5-FNAM and methyl-5-bromonicotinate were about equally inhibitory. 5-FNA did not inhibit this organism.

TABLE 2. *Reversal of activity of 5-fluoronicotinic acid against Streptococcus sp. 1820 by nicotinic acid*

5-Fluoronicotinic acid	Nicotinic acid* (μg/ml)			
	0.02	0.06	0.2	0.6
μg/ml				
0.5	3	6	6	6
0.2	3	5	5	23
0.1	3	14	20	82
0.05	7	38	87	107
0.00	97	108	107	117

* Results expressed as Klett-Summerson readings.

One part of nicotinic acid reversed the inhibitory action of 1,000 parts of 5-FNAM.

Tests with E. coli strain 105. Of 11 analogues of nicotinic acid tested, 7 showed 50% or more inhibition. All compounds tested had substitutions in the 5 position. Six of the compounds were reversed by nicotinic acid. 5-FNA and 5-FNAM were the most inhibitory compounds. One part of nicotinic acid reversed the inhibitory action of 100 parts of 5-FNA or 1,000 parts of 5-FNAM.

Tests with E. coli strain ATCC 8723b. Of 20 analogues of nicotinic acid tested against this nicotinic acid-requiring mutant of *E. coli*, 10 showed 50% or more inhibition. Six of the inhibitors had substitutions in the 5 position; four of them, nicotinhydroxamic acid, 5-thiazole carboxamide, 6-aminonicotinic acid, and 4-

TABLE 3. *Effect of 5-fluoronicotinic acid and related compounds against Streptococcus pyogenes infection in mice*

Compound*	LD_{50}	Avg days of survival†	No. of survivors/ no. on test
6-Aminonicotinic acid	69	1.6	0/5
5-Bromonicotinamide	69	1.0	0/5
5-Bromonicotinic acid	69	1.0	0/5
5-Chloronicotinic acid	69	1.2	0/5
5,6-Diaminonicotinic acid	69	1.6	0/5
5,6-Dichloronicotinic acid	69	2.2	1/5
5-Fluoro-3-cyanopyridine	69	6.2	4/5
	24	2.2	0/8
5-Fluoro-*N'*-dimethylamino-methylnicotinamide	69	7.0	5/5
	24	7.0	8/8
5-Fluoronicotinamide (5-FNAM)	194	7.0	5/5
5-Fluoronicotinamide-*N*-oxide	69	5.2	2/5
	194	4.4	2/5
	24	6.4	7/8
5-Fluoronicotinhydroxamic acid	69	6.0	4/5
5-Fluoronicotinic acid hydrazide	69	5.4	2/5
	24	7.0	8/8
5-Fluoronicotinic acid-*N*-oxide	69	2.0	0/5
	24	5.2	7/8
5-Fluoroquinolinic acid, monosodium salt	194	1.2	0/5
5-Hydroxynicotinic acid	69	1.6	0/5
5-Iodonicotinamide	69	1.2	0/5
Methyl-5-bromonicotinate	69	1.4	0/5
5-Methylthionicotinamide	69	3.0	0/5
	24	3.6	2/8
5-Thiazole carboxylic acid	69	1.4	0/5
Thioisonicotinamide	69	1.0	0/5

* Treatment consisted of two 83 mg/kg doses administered subcutaneously at 1 and 5 hr after infection.

† Test was terminated on 7th day after infection.

nitronicotinic acid-*N*-oxide, did not. The inhibition by seven compounds was reversed by nicotinic acid; the inhibition by two compounds was not reversed by nicotinic acid. The only compound with a substitution in the 5 position of the pyrimidine ring which was not reversed by nicotinic acid was 5-phenylnicotinic acid; compounds not having this substitution, which were reversed, were 5-thiazole carboxamide and 6-aminonicotinic acid.

Tests with L. plantarum strain ATCC 8014. Of the 26 compounds tested for inhibition, only 3 resulted in 50% or more inhibition. Two compounds were tested for reversal of inhibition by nicotinic acid and found reversible. This organism was the least susceptible of the organisms used to study the inhibition by nicotinic acid analogues.

In vivo tests. Of 21 compounds tested in mice for activity against *S. pyogenes*, 9 compounds showed activity. Table 3 summarizes the findings with 20 compounds. A significant number of animals were protected by 5-fluoro-3-cyanopyridine, 5-fluoro-*N*-dimethylaminomethylnicotinamide, 5-FNAM, 5-fluoronicotinamide-*N*-oxide, 5-fluoronicotinic acid-*N*-oxide, 5-fluoronicotinhydroxamic acid, and 5-fluoronicotinic acid-hydrazide. One compound showing only significant lengthening of life was 5-methylthionicotinamide. 5-FNA, tested against 645 and 420 LD_{50} levels of *S. pyogenes*, had ED_{50} values of 58 and 57 mg/kg × two treatments subcutaneously, respectively. When 20 mg/kg × two treatments of nicotinic acid were simultaneously administered in another location, 200 mg/kg × two treatments of 5-fluoronicotinic acid gave no protection.

Eight compounds were tested against 2 LD_{50} and 20 LD_{50} of *E. coli*, 10 LD_{50} of *P. vulgaris*, and 58 LD_{50} of *P. aeruginosa*. Six of these compounds had shown activity against *S. pyogenes* but none showed significant activity against any of these organisms (Table 4).

DISCUSSION

Compounds (43) related to nicotinic acid were tested for inhibition of *Streptococcus* sp. and several additional test organisms. Of these compounds, 32 had a substitution in the 5 position of the pyridine ring. Of these, 20 were inhibitory for at least one organism. Other analogues (11) with no substitution in the 5 pyridine were tested. Of these, nine were inhibitory for at least one organism.

5-FNA was the most inhibitory compound examined against *Streptococcus* sp., *E. coli*, and *L. plantarum*, but failed to inhibit the growth of *S. aureus*. On the other hand, 5-FNAM was the most active compound against *S. aureus* examined.

TABLE 4. *Effect of 5-fluoronicotinic acid and related compounds against Escherichia, Proteus, and Pseudomonas infections in mice*

Compound	Treatment*	No. of survivors/no. on test			
		E. coli		*P. vulgaris*	*P. aeruginosa*
	mg/kg	(20 LD_{50})	(2 LD_{50})	(10 LD_{50})	(58 LD_{50})
Control		1/5	2/5	0/5	0/5
5-Fluoro-3-cyanopyridine	83	0/5	1/5	0/5	0/5
5-Fluoro-*N'*-dimethylaminomethylnicotinamide	83	0/5	2/5	0/5	0/5
5-Fluoronicotinamide (5-FNAM)	83	2/5	1/5	0/5	1/5
5-Fluoronicotinamide-*N*-oxide	83	0/5	0/5	0/5	0/5
5-Fluoronicotinhydroxamic acid	71			0/5	0/5
5-Fluoronicotinic acid hydrazide	83	2/5	2/5	0/5	0/5
5-Fluoronicotinic acid-*N*-oxide	83	0/5	1/5		
(LD_{50})		(316)		(475)	(6,800)
5-Fluoroquinolinic acid, monosodium salt	83	0/5		0/5	0/5

* The treatment was administered subcutaneously twice, 1 and 5 hr after infection.

There was considerable variation between the different organisms in their sensitivity to the compounds studied. *L. plantarum*, although used for the microbiological assay for nicotinic acid, was inhibited by only 3 of the 25 compounds tested against it. *E. coli* strain 105 on the other hand requires no external source of nicotinic acid and was very sensitive to many of the compounds. Sensitivity to these compounds is not correlated completely to the requirement for nicotinic acid. Inhibition was quite specific; e.g., 5-bromonicotinamide and 5-thiazole carboxamide inhibited *E. coli* but not *Streptococcus*, but 5-FNAM and 4-aminonicotinic acid inhibited *Streptococcus* and not *E. coli*.

When the greater activity of 5-FNA and 5-FNAM was first detected, it was hoped that other highly active compounds might be found. Replacing the 5-fluoro radical with an amino, bromo, chloro, dimethylamine, hydroxy, iodo, methyl, or phenyl radical resulted in much less or no inhibition with *Streptococcus*. When the fluoro radical was moved to the 6 position, no inhibition was detected. In contrast, the amino in the 5 position showed very slight activity (5-aminonicotinic acid·HCl) but in the 4 or 6 position showed definitely more activity. Modification of the 3-carboxylic acid group on the pyridine ring acted to neutralize, at least in part, the inhibition produced by the 5-fluoro radical. Although thionicotinamide inhibits the growth of *Streptococcus*, the 5-fluorothionicotinamide had no increased inhibitory power. The 5-fluoro substituent contributed nothing to the activity. Of all the substitutions made on the 5 position of the pyridine ring, the fluoro radical would take the least space, having a normal covalent radius of 0.64°.

Other modifications in the 3 position were: aceto, carbomethoxy, *N'*-carboxymethylcarbamoyl, cyano, *N*-dimethylaminomethylcarbamoyl, dimethylcarbamoyl, hydrazinocarbamoyl, hydroxyaminocarbamoyl, and thiocarbamoyl. The activity of 5-FNA or 5-FNAM was decreased with any of these changes in the 3 position of the pyridine ring. Likewise, the formation of the *N*-oxide of the 5-FNA or 5-FNAM resulted in loss of activity.

Sufficient quantities of 21 compounds were available for testing against *S. pyogenes* strain C-203 in mice. Eleven compounds were active: three saved all animals; seven saved some of the animals, and prolonged their life; one significantly prolonged life. The ED_{50} of 5-FNA was found to be 58 mg/kg × two treatments subcutaneously. The effectiveness of this drug was reversed by simultaneous administration of nicotinic acid. A high level of the 5-FNA (200 mg/kg × two treatments) would not inhibit the growth of the *S. pyogenes* during the simultaneous administration of nicotinic acid (20 mg/kg × two treatments). It would appear that these compounds, all with a substitution in the 5 position of the pyridine ring, are active in

mice. The mg/kg level required for activity does not compare favorably with presently available antibiotics. The reversal of action of 5-FNA in vitro was again found in vivo, but with a difference. In the case of *Streptococcus* in vitro, 4 parts of nicotinic acid were required to reverse the action of 1 part of 5-FNA; with *S. pyogenes* in mice, 1 part of nicotinic acid reversed the action of more than 10 parts of 5-FNA. It would appear probable that the activity of these compounds in vivo was due, at least in part, to an interfering with the metabolic pathway in which nicotinic acid or nicotinamide functions.

None of the eight compounds tested protected mice against *E. coli*, *P. vulgaris*, or *P. aeruginosa*, although seven of these compounds demonstrated activity for *S. pyogenes* in vivo. The growth of two strains of *E. coli* was inhibited in vitro by 5-FNA and related compounds. Fildes (1940) found that 10 strains of *Proteus* required nicotinic acid for growth. *Pseudomonas* normally requires no external sources of nicotinic acid. These compounds had no activity against any of these organisms in mice, even though with *E. coli* and *P. vulgaris* there were grounds for suspecting that they would be vulnerable to an antinicotinic acid compound. It should be borne in mind that there may be considerable strain specificity by gram-negative bacteria to antimetabolite drugs, and only one strain of each of three species has been tested. These negative results do not preclude activity of these compounds against other strains of these organisms.

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